## REMARKS/ARGUMENTS

The Office Action mailed February 25, 2004, has been received and reviewed. Claims 1-8 are currently pending in the application. Claim 9 has been withdrawn from consideration, in view of the previous restriction requirement. Claims 1-8 stand rejected. Applicants have amended claims 1 and 5. New claims 10-21 have been added. In view of the above amendment and the following remarks, Applicants believe all claims are now in condition for allowance, and respectfully request reconsideration and withdrawal of the outstanding rejections.

### Claim Amendment

Claims 1 and 5 have been amended to more particularly define the subject matter of the claimed invention.

Specifically, claims 1 and 5 are amended to include a reference to the nucleic acid "sequence," so as to provide antecedent basis for the term "sequence" in subpart (a). Support for this limitation is found in the claim itself, which refers to the "sequence," as well as in the Specification at page 2, last line.

Claims 1 and 5 are also amended to reflect that the alignment points may include "one or more" internal peaks. Support for this limitation is found in the Specification at, for example, page 7, lines 3-5, lines 20-22; page 8, lines 7-23.

Claims 1 and 5, subpart (a), are also amended to include the term "relative" in reference to the position of the alignment point with respect to the sequence as a whole. Support for this term is found in the Specification at page 6, line 22.

Claims 1 and 5, subpart (a), are also amended to delete the reference to the alignment point "having" a reference position number, and adding the term "assigning" to indicate that the alignment points are assigned a reference position number as part of the method.

Claims 1 and 5, subpart (b), are amended to delete the phrase "and sequence position numbers being assigned to," which is redundant language, add the word "that" and pluralize the word "maximizes," for grammatical correctness.

Claims 1 and 5, subpart (b), are amended to delete the word "matching," to which the Examiner has objected on grounds that it lacks antecedent basis, and which the Applicant

considers in any event to be redundant in view of the remaining language that recites a method that results in matching of reference position numbers for the alignment points and sequence position numbers of the data traces to a base of the same type.

Claim 3 is amended to change the dependency from claim 2 to claim 1.

Claims 10 is newly added, which recites a method that requires only "one or more alignment points corresponding to an internal peak associated with internal bases that are highly conserved in the target nucleic acid." Support for a method that requires only "one or more" internal alignment points is found in the Specification at page 7, lines 25-27 through page 8, line 6.

Claims 11-13 are newly added claims that depend from claim 10.

Claim 14 is newly added, which also depends from claim 10, and is directed to a method that further comprises "alignment points selected from the group consisting of a primer peak associated with unextended primer, a full-length peak associated with full length product produced during a cyclic primer extension reaction with two primers."

Claims 15-17 are newly added and depend from claim 14.

Claims 18-21 are newly added, and depend from claims 1, 5, 10 and 14, respectively. These claims are directed to an optional step of "determining the average peak spacing interval between alignment points and assigning sequence position numbers to peaks occurring at said intervals, wherein the sequence position numbers are used for aligning the data traces based on the assigned sequence position numbers." This step recites features not believed to be disclosed in the prior art. Support for these claims is found in the Specification at page 10, line 24 through page 11, line 15.

**Restriction Requirement**

The Examiner has maintained that the process of the present invention can be practiced with any of a number of different sequencing devices. Applicants accordingly withdraw the traversal of the restriction requirement and withdraw claim 9 from consideration, with reservation of the right to prosecute claim 9 in a later-filed divisional application.

**Objections**

The Examiner has objected to the title of the invention on grounds that the claims directed to the apparatus have been withdrawn. Applicants have accordingly amended the title to remove the reference to an apparatus.

**Information Disclosure Statement**

The Examiner has indicated that Tibbetts et al. has not been considered because Form 1449 does not provide publication information, such as publisher and publication date. Applicants submit herewith a new Form 1449 providing the required information.

**Claim Rejection – 35 U.S.C. 112, Second Paragraph**

Claims 1-8 have been rejected under 35 U.S.C. 112, second paragraph, on grounds that the term "sequence" has no antecedent basis. Claims 1 and 5 have been amended to provide such antecedent basis in the claim preamble.

Claims 1-8 have also been rejected under 35 U.S.C. 112, second paragraph, on grounds that the term "matching reference position number" in claims 1 and 5 lack antecedent basis. Applicant has accordingly deleted the term "matching," which is superfluous in view of the existing language that describes the process of "matching" the reference position numbers for the alignment points and sequence position numbers of the data traces to a base of the same type.

**Claim Rejection – 35 U.S.C. 103(a)**

Before addressing the substantive issues relating to the claim rejections under 35 U.S.C. 103(a), Applicants wish to summarize the key elements of the claimed invention, which may assist in clarifying the differences between the claims and the prior art relied on by the Examiner.

The present invention is directed to a method for alignment of a plurality of data traces indicative of the positions of a plurality of nucleic acid base types in a target nucleic acid sequence. It is important to note that alignment of data traces is a step that is performed prior to base calling (i.e., assembling the correct order of a polynucleotide sequence). Alignment of *data traces* is therefore different from alignment of *nucleotide sequences with each other* or with the original data trace for comparison or confirmation of the nucleotide sequence that has already been previously assembled by alignment of a data trace and base calling. An important aspect of

the claimed method is the initial selection of one or more alignment points, based on the known nucleic acid sequence (i.e., not derived from the data trace). The alignment point may be a single or multiple internal alignment point based on a highly conserved region. In an alternate embodiment, the alignment points are selected from among the (i) primer peak, (ii) the full-length peak, and (iii) internal peaks representing highly conserved bases. In preferred embodiments, the internal peak alignment points are heterogeneous nucleotide multiplets. Reference position numbers are assigned to these alignment points, reflecting the known position of the alignment point relative to the rest of the sequence. These reference position numbers are used as a reference point in the next step when comparing position numbers that are assigned to peaks in the data traces. To each peak in the experimental data trace, a sequence position number is assigned so as to maximize the number of times that the sequence position number of the data trace is assigned to the same base as the reference position number of the alignment points. Optionally, the claimed method may also include a step of determining the average peak spacing interval between alignment points and assigning a sequence position number to peaks occurring at the intervals. The data traces are then aligned based on the assigned sequence position numbers.

The method of the present invention described above has been shown to result in significantly improved accuracy of base-calling, particularly where there is gross misalignment problems. Prior art methods of base calling are inherently incapable of processing data traces with gross misalignment between them. It is not uncommon, particularly when base calling is required in conjunction with the use of 2-color sequencers that use up to 4 traces obtained from different physical lanes of the slab gel or different capillaries for there to be a shift between traces exceeding 20 – 30 bases. Alignment of data traces with such shift without *a priori* information is highly complicated, and algorithms used prior to the present invention are not always capable of successfully resolving such data traces. The method of the present invention, which utilizes stable conserved peaks (such as primer peaks, final full-length peaks and/or conserved regions of sequence), however, has been shown to enable successful and accurate alignment of data traces having initial misalignment as high as 150 bases (see, e.g., Figures 10 and 11 of the specification), which is a highly significant improvement over the prior art that was not expected, nor could have been predicted *a priori*.

As shown in detail below, the prior art relied on by the Examiner does not teach or suggest the method of the present invention, and is not capable of achieving the same results as the present invention. Because the prior art relied on by the Examiner, either alone or in combination, fails to disclose the recited limitations of the claims, the rejections should be withdrawn and the claims allowed.

In the only rejection based on prior art, the Examiner has rejected claims 1-8 under 35 U.S.C. 103(a) as being unpatentable over Giddings et al. taken with Ewing et al.

With respect to Giddings et al., the Examiner states that Giddings discloses a method of analyzing trace data from a DNA sequencing device which uses the primer peak and the farthest minimum located from the primer peak. The Examiner further states that Giddings discloses editing of the base-calls and alignment of bases. Further, the Examiner states that Giddings discloses use of multiplets of nucleic acid molecules.

The present invention is distinguishable over the teachings of Giddings for the following reasons. First, Giddings does not disclose the use of the primer peak as a reference point for alignment of data traces. Giddings describes detection and use of the primer peak for two purposes – (1) for *deletion* of the data points preceding the primer peak, and (2) identification of the subsequent minimum prior to the first resolved peak and *deletion* of all data before this minimum (including the primer peak data itself). Specifically, Giddings discloses that the primer peak "is useful for later processing to *remove all data before the primer peak*" (page 652, column 1, lines 9-10), and that "the routine then finds the subsequent minimum in each of the four channels from the location of this maxima, and *deletes all data before the farthest minima* of the four channels from the location of the maximum" (page 652, column 1, line 21, through column 2, line 3). The "minimum" to which Giddings refers is the deep (or the lowest) point between the primer peak and the first resolved sequence peak. The primer peak is removed from the data set because its large size, if left in the data, would complicate calculations and reduce reliability and stability of the results. Consequently, the method of Giddings, as stated on page 652 (column 2, line 1), deletes all the data points in all four channels preceding the farthest minimum after the primer peak. Significantly, Giddings concedes that their method "does not work for dye terminator data, as there is no primer peak to detect" (page 652, column 2, lines 6-7). Thus, Giddings uses the primer peak solely for the purpose of deleting all data before the minimum, including the primer peak itself.

In contrast, the method of the present invention not only permits retention of the primer peak in the data, but advantageously makes use of the primer peak as a reference point for trace alignment, resulting in improved accuracy of base-calling. In describing the use of the primer peak alignment point (which is also applicable to the final peak and internal conserved peaks), claims 1 and 5 recite "alignment points being selected from the group consisting of a primer peak," "assigning to said alignment points a reference position number reflecting the relative position of the alignment point with respect to the sequence as a whole," and use of the reference position numbers in "assigning a sequence position number to each peak . . . to maximize the number of times that the sequence position number and the reference position number are assigned to a base of the same type," and finally "aligning the data traces. The present claims allow alignment based on the conserved areas of the sequence and do not require use of the primer peak, although the primer peak may (but is not required to) be used as one of the conserved areas as its length is predetermined and remains constant for a given type of sample. In short, Giddings fails to disclose claims limitations that recite use of a primer peak as an alignment point in relation to any other alignment point, assigning a reference position to the primer peak, and use of the primer peak in assigning bases.

Second, the present invention is also distinguishable over Giddings because Giddings does not disclose the particular method of aligning two or more data traces recited in the claims. The Examiner states that Giddings describes "consensus (alignment)," and cites to page 663, column 2, lines 60 to page 664, column 1, line 13. The relevant section of Giddings, however, specifically states that "each *base in the final consensus* is manually verified with the individual traces from which the consensus was determined." The process described by Giddings thus relates to alignment of a consensus sequence (i.e., the string of letters describing the nucleotide sequence obtained *after* base calling is already completed, but not original data traces) with individual data traces, as a means for assessing the quality of the base calling. This is different from the process of the present invention, which aligns one data trace with another data trace for the purpose of the making initial base-call itself. Specifically, the limitations in claims 1 and 5, subpart (c) recite "aligning data traces," which "alignment" process takes place *prior* to base calling. Giddings does not therefore teach the claimed method of "aligning data traces," as recited in the claims.

Third, the present invention is distinguishable over Giddings because Giddings does not disclose use of alignment points based on "highly conserved" internal regions, or that such internal alignment points can be "heterogeneous multiplets." Giddings contains no teaching or hint of identifying a "highly conserved" internal region as an alignment point. The Examiner contends that Giddings is "directed to multiplets of nucleic acid molecules," but the "multiplets" used by Giddings are completely different than the multiplets of the present invention. Giddings discloses a software tool for performing background subtraction to remove background signals. This tool searches for minimum signal intensity in windows of size *n*, which "window *n* must be set larger than the width of any of the expected peaks (including multiplet peaks) to avoid removal of desired signal data" (page 653, column 1, lines 6-22). The "multiplets" of Giddings thus represent an arbitrary window of size *n*, which is the region within which one determines minimum signal intensity that is then subtracted as background noise. In contrast, the "multiplets" recited in the present claims are used for an entirely different purpose – namely, as an alignment "lock" from which the location of other nucleotides is calculated. Moreover, in a preferred embodiment of the present invention, the multiplets are "heterogeneous." In addition, the "internal peak alignment points" are assigned "a reference position number reflecting the relative position of the alignment point with respect to the sequence as a whole," which is then used "to maximize the number of times that the sequence position number and the reference position number are assigned to a base of the same type." Thus, while the "multiplets" of Giddings are used for subtracting background noise in discrete blocks, the "multiplets" of the present invention are used as a calibration point, which is an entirely different purpose. In view of these differences, Giddings does not teach or suggest the use of internal peak alignment points that can be "heterogeneous" multiplets.

The rejection of claims 1-8 under 35 U.S.C. 103(a) is also predicated on the teachings of Ewing et al. For the reasons detailed below, Ewing does not disclose the method of the present invention, and does not disclose the features of the claimed method that are lacking in the Giddings publication. The Examiner states that Ewing discloses a method of base-calling comprising determining the idealized peak locations, the correct number of bases, the observed peaks, matching the observed peaks to the predicted peak locations, and assigning the respective peaks with 1 of the 4 bases to determine a base sequence for the trace. Ewing thus attempts to match *all* observed peaks with *all* predicted peak locations. In contrast, the claims of the present

invention are directed to a method that selectively uses highly conserved regions (such as the primer peak, the final full-length peak and internal peaks corresponding to highly conserved nucleotides) as reference points to facilitate more accurate alignment of other peaks. The claims of the present invention require selecting three or more alignment points "selected from the group consisting of a primer peak associated with unextended primer, a full-length peak associated with full length product produced during a cyclic primer extension reaction with two primers, and one or more internal peaks associated with internal bases that are highly conserved in the target nucleic acid, and assigning to said alignment points a reference position number reflecting the relative position of the alignment point with respect to the sequence as a whole." Ewing discloses nothing about using the primer peak as a reference point, using a full length peak as a reference point, or using an internal peak associated with a highly conserved region as a reference point, as required by the claims of the present invention. Nor does Ewing disclose assigning "reference position numbers" that reflect "the relative position of the alignment point with respect to the sequence as a whole," which is also required by the claims. As shown in the specification (see, e.g., Figures 10 and 11), the selection and use of selected alignment points allows accurate alignment not achieved by the methods of the prior art.

Furthermore, Ewing does not disclose "assigning a sequence position number to each peak in each of the plurality of data traces," nor does Ewing disclose assigning "sequence position numbers ... to maximize the number of times that the sequence position number and the reference position number are assigned to a base of the same type," as require by the claims. The Examiner states that Ewing teaches "comparing the minimum and maximum values of peaks to maximized [sic] the number of times a peak value is assigned to the predicted peak," but comparing minimum and maximum values of peaks is not what the claims of the present invention recite. The claims of the present invention recite a process in which sequence position numbers are assigned to peaks in a data trace so as to maximize the number of times the sequence position number and reference position number are matched to a base of the same type. Whatever Ewing discloses, it is entirely different from and unrelated to the present claims.

In combining the Giddings and Ewing references, the Examiner states that one skilled in the art "would have been motivated by the improvements disclosed by Ewing et al. . . . in a method for analyzing trace data from DNA sequencing apparatus as taught by Giddings et al." As explained in detail above, however, neither Giddings nor Ewing disclose selecting alignment

points (all points are used; therefore, none are "selected"). Neither Giddings nor Ewing disclose using a primer peak as an alignment point. Neither Giddings nor Ewing disclose using a full-length peak associated with full length product produced during a cyclic primer extension reaction with two primers. Neither Giddings nor Ewing disclose using internal peaks associated with highly conserved regions in the target nucleic acid. Neither Giddings nor Ewing disclose assigning to these alignment points a reference position number reflecting the relative position of the alignment point with respect to the sequence as a whole. Neither Giddings nor Ewing disclose assigning a sequence position number to each peak in each of the plurality of data traces to maximize the number of times that the sequence position number and the reference position number are assigned to a base of the same type. Neither Giddings nor Ewing disclose aligning the data traces based on the assigned sequence position numbers. Neither Giddings nor Ewing disclose use of heterogeneous multiplets as internal peaks. And neither Giddings nor Ewing disclose determining the average peak spacing interval between alignment points and assigning a sequence position number to peaks occurring at the intervals, wherein the sequence position numbers are used for aligning the data traces based on the assigned sequence position numbers. It is clear that the teachings of Giddings and Ewing do not disclose the key limitations of the present claim, either alone or in combination. The significant gaps between the claimed invention and the Giddings and Ewing references are such that even if the references are combined, they still fail to disclose each and every element of the claimed invention, and do not support the outstanding rejections.

## CONCLUSION

In response to the Examiner's rejections, Applicants have amended the claims to more particularly and more clearly point out the claimed invention, and distinguish the claimed invention over the prior art. Applicants have also amended the Title and Abstract, and have responded to the other objections noted by the Examiner.

In view of the differences between the cited art relied on by the Examiner, and the limitations recited in the claims, Applicants respectfully submit that the cited art neither teaches nor suggests the claimed invention.

For the above reasons, Applicants respectfully request allowance of the claims.

Respectfully submitted,

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